

Application Serial No. 10/665,708
Filed: September 18, 2003

Confirmation No. 6892
Atty. Docket No. GP107-03.DVI
AMENDMENT

IN THE CLAIMS

Claims 1-12 stand withdrawn, and claims 1 and 8-20 have been amended as shown.

1. (Withdrawn - Currently amended) A method of detecting *Mycobacterium* species present in a biological sample, comprising the steps of:

providing a biological sample containing nucleic acid from at least one *Mycobacterium* species comprising a *Mycobacterium* 16S ribosomal RNA (rRNA) or DNA encoding a *Mycobacterium* 16S rRNA;

amplifying the *Mycobacterium* 16S rRNA or *Mycobacterium* DNA encoding the *Mycobacterium* 16S rRNA in an in vitro nucleic acid amplification mixture comprising at least one polymerase activity, and a combination of at least two primers having sequences selected from the group consisting of a first primer of SEQ ID NO:11 and a second primer that is an oligonucleotide consisting of 19 to 25 bases that contains contiguous bases 1 to 18 of SEQ ID NO:24 and optionally three to seven bases 5' to the contiguous bases 1 to 18 of SEQ ID NO:24 and/or optionally one base 3' to the contiguous bases 1 to 18 of SEQ ID NO:24 SEQ ID NO:1 to SEQ ID NO: 34, SEQ ID NO:37 and SEQ ID NO:38 to produce amplified *Mycobacterium* nucleic acid; and

detecting the amplified *Mycobacterium* nucleic acid by detecting a label associated with the amplified *Mycobacterium* nucleic acid.

2. (Withdrawn - Original) The method of Claim 1, further comprising in the steps of:

adding to the biological sample at least one capture oligonucleotide that specifically hybridizes to the *Mycobacterium* 16S rRNA and an immobilized nucleic acid that hybridizes to the capture oligonucleotide under hybridizing conditions to produce a hybridization complex;

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and

separating the hybridization complex from other components of the biological sample before the amplifying step.

3. (Withdrawn - Original) The method of Claim 1, wherein the amplifying step amplifies 16S rRNA or DNA encoding 16S rRNA from *M. tuberculosis* or a *Mycobacterium* other than *tuberculosis* (MOTT) species.

4. (Withdrawn - Original) The method of Claim 1, wherein the amplifying step amplifies 16S rRNA or DNA encoding 16S rRNA from *M. abscessus*, *M. africanum*, *M. asiaticum*, *M. avium*, *M. bovis*, *M. celatum*, *M. chelonae*, *M. flavescens*, *M. fortuitum*, *M. gastri*, *M. gordoneae*, *M. haemophilum*, *M. intracellulare*, *M. interjectum*, *M. intermedium*, *M. kansasii*, *M. malmoense*, *M. marinum*, *M. non-chromogenicum*, *M. paratuberculosis*, *M. phlei*, *M. scrofulaceum*, *M. shimodei*, *M. simiae*, *M. smegmatis*, *M. szulgai*, *M. terrae*, *M. triviale*, *M. tuberculosis*, *M. ulcerans* or *M. xenopi*.

5. (Withdrawn - Original) The method of Claim 1, wherein the detecting step uses at least one probe that hybridizes specifically to the amplified *Mycobacterium* nucleic acid.

6. (Withdrawn - Original) The method of Claim 5, wherein the detecting step uses at least one labeled probe that hybridizes specifically to the amplified *Mycobacterium* nucleic acid.

7. (Withdrawn - Original) The method of Claim 5, wherein the detecting step uses a plurality of probes that hybridize specifically to the amplified *Mycobacterium* nucleic acid.

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8. (Withdrawn - Currently amended) The method of Claim 1, wherein the amplifying step uses a combination of at least a first primer and a second primer, wherein the first primer is consists of SEQ ID NO:11 selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:12, and the second primer is selected from the group consisting of SEQ ID NO:21, SEQ NO:22, SEQ ID NO:23 and SEQ ID NO:24 SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 and SEQ ID NO:38.

9. (Withdrawn - Currently amended) The method of Claim 8, wherein the amplifying step uses a combination of at least a first primer and a second primer, wherein the first primer is selected from the group consisting of SEQ ID NO:7 to SEQ ID NO:12, and the second primer is consists of SEQ ID NO:21, selected from the group consisting of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 and SEQ ID NO:38.

10. (Withdrawn - Currently amended) The method of Claim 8, wherein the amplifying step uses a combination of at least a first primer and a second primer consists of SEQ ID NO:22, selected from the group consisting of:

- the first primer having the sequence of SEQ ID NO:7, and the second primer having the sequence of SEQ ID NO:13;
- the first primer having the sequence of SEQ ID NO:7, and the second primer having the sequence of SEQ ID NO:14;
- the first primer having the sequence of SEQ ID NO:7, and the second primer having the sequence of SEQ ID NO:15;
- the first primer having the sequence of SEQ ID NO:7, and the second primer having the sequence of SEQ ID NO:16;
- the first primer having the sequence of SEQ ID NO:8, and the second primer having the

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~~sequence of SEQ ID NO:13;~~

~~the first primer having the sequence of SEQ ID NO:8, and the second primer having the sequence of SEQ ID NO:14;~~

~~the first primer having the sequence of SEQ ID NO:8, and the second primer having the sequence of SEQ ID NO:15;~~

~~the first primer having the sequence of SEQ ID NO:9, and the second primer having the sequence of SEQ ID NO:13;~~

~~the first primer having the sequence of SEQ ID NO:9, and the second primer having the sequence of SEQ ID NO:14;~~

~~the first primer having the sequence of SEQ ID NO:9, and the second primer having the sequence of SEQ ID NO:15;~~

~~the first primer having the sequence of SEQ ID NO:10, and the second primer having the sequence of SEQ ID NO:16;~~

~~the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:13;~~

~~the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:16;~~

~~the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:17;~~

~~the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:18;~~

~~the first primer having the sequence of SEQ ID NO:11, and the second primer having the sequence of SEQ ID NO:19;~~

~~the first primer having the sequence of SEQ ID NO:11, and the second primer having the~~

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~~sequence of SEQ ID NO:20, and~~

~~the first primer having the sequence of SEQ ID NO:12, and the second primer having the sequence of SEQ ID NO:15.~~

11. (Withdrawn - Currently amended) The method of Claim 8, wherein ~~the amplifying step uses a combination of the first primer having the sequence of SEQ ID NO:11, and the second primer consists of SEQ ID NO:23, having the sequence of SEQ ID NO:16, SEQ ID NO:30 or SEQ ID NO:37.~~

12. (Withdrawn - Currently amended) The method of Claim 8, wherein ~~the amplifying step uses a combination of the first primer second primer consists of SEQ ID NO:24, having the sequence of SEQ ID NO:11, and two second primers having the sequences SEQ ID NO:16 and SEQ ID NO:37.~~

13. (Currently amended) A composition for amplifying in an in vitro amplification reaction a *Mycobacterium* 16S rRNA sequence or a DNA encoding 16S rRNA, comprising a combination of at least two oligonucleotides, wherein a first oligonucleotide contains a promoter sequence and a sequence that hybridizes to a *Mycobacterium* 16S rRNA or DNA sequence, and a second oligonucleotide is an oligonucleotide consisting of 19 to 25 bases ~~that contains~~, containing 18 contiguous bases 1 to 18 of SEQ ID NO:24 and optionally three to seven bases 5' to the 18 contiguous bases 1 to 18 of SEQ ID NO:24 and/or optionally one base 3' to the contiguous bases 1 to 18 of SEQ ID NO:24.

14. (Currently amended) The composition of Claim 13, wherein the composition comprises:
at least one first oligonucleotide ~~having the sequence consisting of SEQ ID NO:11~~ any one of SEQ ID NO:1 to SEQ ID NO:12, and
at least one second oligonucleotide ~~having the sequence of any one of consisting of SEQ ID~~

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~~NO:21, SEQ ID NO:22, SEQ ID NO:23 or SEQ ID NO:24, SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 or SEQ ID NO:38.~~

15. (Currently amended) The composition of Claim 14, wherein the composition comprises:

~~the at least one first oligonucleotide containing the sequence of any one consisting of SEQ ID NO:11 to SEQ ID NO:12, and~~

~~the at least one second oligonucleotide consisting of SEQ ID NO:21, containing the sequence of any one of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 or SEQ ID NO:38.~~

16. (Currently amended) A kit containing one or more oligonucleotides having a sequence selected from the group consisting of ~~SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, SEQ ID NO:1 to SEQ ID NO:34, SEQ ID NO:37 and SEQ ID NO:38.~~

17. (Currently amended) The kit of claim 16, further containing an oligonucleotide consisting of SEQ ID NO:11.

~~at least one first oligonucleotide having the sequence of any one of SEQ ID NO:1 to SEQ ID NO:12, and~~

~~at least one second oligonucleotide having the sequence of any one of SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 or SEQ ID NO:38.~~

18. (Currently amended) The kit of claim 17, containing

~~at least one a first oligonucleotide consisting of SEQ ID NO:11 containing the sequence of any one of SEQ ID NO:7 to SEQ ID NO:12, and~~

~~at least one second oligonucleotide containing the sequence of any one of consisting of SEQ ID~~

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NO:21, SEQ ID NO:22, or SEQ ID NO:23, SEQ ID NO:13 to SEQ ID NO:34, SEQ ID NO:37 or
SEQ ID NO:38.

19. (Currently amended) The composition of Claim 14, wherein the composition comprises:

the at least one first oligonucleotide consisting of SEQ ID NO:11, and
the at least one second oligonucleotide consisting of SEQ ID NO:23.

20. (Currently amended) The composition of Claim 14, wherein the composition comprises:

the at least one first oligonucleotide consisting of SEQ ID NO:11, and
the at least one second oligonucleotide consisting of SEQ ID NO:24.